# CHANGES IN THE ACTIVITY OF SUCCINIC DEHYDROGENASE AND CYTOCHROME OXIDASE IN EXPERIMENTAL MANGANESE POISONING

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The hazards of the increasing incidence of occupational manganese intoxications among workers engaged in mining and industrial production pose the problem of further researches into manganese poisoning.

Recently, the interest has been focused on the study of manganese effect on certain enzyme systems, containing metal ions and active SH-groups, liable to eventual replacement or $Mn^{2+}$ binding (3, 11).

Succinic dehydrogenase (succinate-oxidoreductase, E. C. — 1,1, 3,1) is one of the essential redox-enzymes in the cycle of tricarbonic acids, and the primary ring in the chain of transfer of electrons to oxygen (2). Besides flavin, in the composition of its prosthetic groups, four $Fe^{2+}$ atoms also participate, in all likelihood bound to the SH-groups of the protein part of the enzyme.

Cytochrome oxidase (cytochrome C:$O_2$-oxidase, E. C. — 1,1, 3,1) is an essential linkage in the oxidation processes of the organism, and plays an exceptionally important role in cellular respiration (2). It contains one iron atom, bound in the form of hemochromogen.

The composition and behaviour of the two enzymes are presumably influenced by manganese which has some chelating properties, dependent upon its concentration in the biological substrates. Literature data concerning researches in this line are very scant. Jonderko and co-authors (13, 14, 15, 16) found a reduced activity of hepatic succinic dehydrogenase and cytochrome oxidase in rabbits following a relatively short poisoning period without ensuing recovery; moreover, no relationship was established between the concentration of the metal and enzyme activity.

Having in mind the practical bearing of the manganese poisoning problem, the role and behaviour of manganese in enzyme-catalytic processes, as well as the properties and importance of the mentioned enzymes, we made it our aim:

— to trace the changes in the activity of succinic dehydrogenase and cytochrome oxidase, in dynamics, in the course of experimentally induced manganese poisoning, and

— to assay the possibility of using these changes in the early diagnosis of manganese intoxication.

## Material and Method

The changes in succinic dehydrogenase and cytochrome oxidase under conditions of experimental manganese intoxication were studied in 120 white male rats, with average weight about 130 g, divided up into groups as shown in Table 1.

Table 1

| Groups \ Experim. days | 20 | 40 | 60 | 120 |
|---|---|---|---|---|
| Controls-number | 10 | 10 | 10 | 10 |
| Poisoned-number | 20 | 20 | 20 | 20 |

The animals experimented upon were poisoned every other day with a $MnCl_2.4H_2O$ solution per os, in the first 20 days — with $Mn^{2+}$ at 30 mg/kg dose, and thereafter, till the end of the experiment — with 100 mg/kg $Mn^{2+}$.

Succinic dehydrogenase activity was determined in 10 per cent brain and liver homogenate after the method of Kun and Abood (8). Formazan produced from 1 ml homogenate for 120 min, at 37° C, was calculated in mcg. Besides in the organs indicated above, cytochrome oxidase activity was also determined in 10 per cent blood hemolysate according to the method of Vernon (1), as modified by the research alimentation laboratory with the Higher Medical Academy «S. M. Kirov». The activity was calculated in mg indophenol, obtained from 1 ml homogenate, resp. hemolysate, for 30 minutes at 20° C.

## Results and Discussion

Experimentally induced manganese poisoning reduces the activity of brain and liver succinic dehydrogenase and cytochrome oxidase (Table 2).

The fall of succinic dehydrogenase becomes statistically reliable ($p=0.05$) after the 20th post-intoxication day. The duration of poisoning and the increase of dose intensify enzyme disorders, and within 60 days the reduction of brain succinic dehydrogenase is nearly 33 per cent, and that of the liver — 63 per cent. The considerably stronger fall of liver succinic dehydrogenase is probably linked to the higher succinic dehydrogenase concentration in the hepatic cells' mitochrondria (7). Our results are in compliance with those reported by Jonderko and co-authors (13, 15, 16).

Within 20 days of poisoning, a slight rise in the activity of brain cytochrome oxidase is noted ($p>0.05$). The confirmation of the latter fact upon increasing the number of animals experimented upon is noteworthy, and probably, it may be attributed to the lower $Mn^{2+}$ doses administered in the initial phase of the experiment. Enhanced activity in the presence of

Table 2

**Changes in the Activity of Succinic Dehydrogenase and Cytochrome Oxidase in Manganese Poisoning**

| Enzymes | Homogenate | Poisoned / Controls | Experimental days: Poisoning 20 | Poisoning 40 | Poisoning 60 | Recovery 120 | r | P |
|---|---|---|---|---|---|---|---|---|
| Succ. dehydr. | brain | 176.5±9.9 | 155.2±4.8 | 134.0±5.4 | 121.0±5.1 | 180.0±4.0 | 0.98 | 0.01 |
| | liver | 611.0±19.2 | 562.5±18.3 | 399.0±19.2 | 228.0±18.8 | 536.2±21.2 | 0.76 | 0.02 |
| Cytochr. oxidase | brain | 74.2±3.8 | 81.0±7.0 | 61.7±8.7 | 41.0±4.1 | 74.3±12.3 | 0.99 | 0.02 |
| | liver | 60.2±1.4 | 59.7±4.2 | 52.3±2.1 | 44.7±2.0 | 60.3±5.9 | 0.70 | 0.05 |
| | blood | 21.5±1.1 | 18.7±1.0 | 16.0±0.9 | 14.7±0.9 | 20.3±2.7 | 0.98 | 0.02 |

small manganese quantities has been reported by other authors, using various enzymes, e. g. in adenosine triphosphatase (6), polynucleotide phosphorylase (4), amino-transferase (16), nicotinamide mononucleotide adenosyltransferase (9), etc.

Increasing the dose and duration of poisoning exerts an inhibitory effect. The following check-up examinations show strongly reduced activity of the brain cytochrome oxidase, and at sixty days it is 55 per cent. After a 60-day-long recovery period, the activity of brain cytochrome oxidase is fully normalized. The changes in enzyme activity in the liver are comparatively milder than those in the brain. A tendency for reduction is noted after the 20th day. At 60 days after the beginning of poisoning, the reduction is 25 per cent, whereas after the recovery period the enzyme regains its normal values.

The changes in blood cytochrome oxidase (30 per cent at 60 days) manifest a close relationship to manganese poisoning ($\gamma=0.98$, $p=0.02$). The decrease in the activity of the enzyme initiates in the very beginning of the experiment (at 20 days $p=0.05$), and thereby undergoes a linear alteration till the end of the poisoning period. Proceeding from the latter findings and considering the low dispersion values with a reference to blood cytochrome oxidase (Table 2), it is assumed that this particular indicator, correlated with the other tests, might be included in the complex set of manganese poisoning indicators.

Against the background of structural peculiarities of succinic dehydrogenase, and with the statement of De Rosa and Fusko (11) concerning the strong affinity of $Mn^{2+}$ for SH groups, and their blocking by $Mn^{2+}$, in mind, we are inclined to explain its changes in the liver and brain by a similar mechanism of interaction.

The results obtained give us sufficient reason to relate the impairment of the nervous system in manganese poisoning to the reduced activity of brain succinic dehydrogenase. Most likely, the latter leads to a slowing down and modification of oxidation processes within the brain.

To clarify the pathomechanism of poisoning, it is of special interest to note the reduction of glutamic acid content in the brain of poisoned animals, already established by us (1), reduction which is connected with a different route of regulating the activity of cerebral succinic dehydrogenase, probably, before a considerable accumulation of manganese in the brain has taken place. Derangements in the citric acid cycle in manganese treatment, related to inhibition of succinic dehydrogenase activity and reduction of glycine concentration in the liver (1), most likely, lead to glycine succinate cycle disorders (Shemin's cycle), directly linked to the synthesis of purine, serine and the like.

Demonstration of the inhibitory effect of manganese on cytochrome oxidase activity is a very complicated and many-sided problem. Our results prove a close relationship between Mn concentration in the biological substrates and enzyme activity. At low concentrations, the oxidation effect of $Mn^{2+}$ is most probably predominating, and is associated with modifications of cellular respiration patterns. At higher concentrations, it is more probable that manganese binds the lateral functional groups of cytochrome oxidase with ensuing formation of chelates (2), which are compounds with reduced reactivity owing to partial changes in the structure of the enzyme, and its physico-chemical properties. A shift of copper and iron ions, participating in the build up of the enzyme, resulting in an overall distrurbance of its structure and activity, is also presumable. The 55 per cent reduction (as compared to controls) in the activity of cerebral cytochrome oxidase at the 60th day does not rule out the above supposition, especially when the large manganese accumulations in the brain are considered (5). A similar $Fe^{2+}$ and $Fe^{3+}$ replacement with manganese has been reported by Perxins in human transferrin (10).

## Conclusions

1. Manganese intoxication causes a reduction of succinic dehydrogenase activity in the brain and liver of white rats. The decrease of the hepatic enzyme activity is considerably stronger than that of the cerebral one.
2. Manganese inhibits the activity of cytochrome oxidase. The most significant changes are recorded in the brain enzyme, next ranking the blood and hepatic one.
3. Changes in blood cytochrome oxidase are statistically reliable, they are closely related to the manganese concentration and could be taken into consideration in working out a laboratory constellation for the early diagnosis of manganese into ication.

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## ИЗМЕНЕНИЯ АКТИВНОСТИ СУКЦИНДЕГИДРОГЕНАЗЫ И ЦИТОХРОМОКСИДАЗЫ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТРАВЛЕНИИ МАРГАНЦЕМ

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РЕЗЮМЕ

Сообщается о снижении активности сукциндегидрогеназы мозга и печени и цитохромоксидазы в крови, мозге и печени у белых крыс, подвергнутых воздействию марганцем — 100 мг/кг $Mn^{2+}$.

Обсуждаются некоторые моменты механизма поражения и некоторые его последствия. Предлагается включить изменения цитохромоксидазы в крови как показатель, одновременно с другими тестами, при ранней диагностике отравления марганцем.